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Prevalence and Phylogenetic Analysis of *Peptostreptococcus* sp. and *Bacteroides* sp. among Ulcerative Colitis, Precancerous Polyps and Colorectal Carcinoma Patients in the Iraqi Population

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ABSTRACT

Ulcerative colitis, precancerous polyps and colorectal carcinoma are associated with dysbiosis of gut microbiota. This study aimed at investigating the prevalence and phylogenetic analysis of *Peptostreptococcus* sp. and *Bacteroides* sp. among Iraqi patients. A total of 100 colorectal biopsy samples were collected from four groups including normal, ulcerative colitis, precancerous polyps and colorectal carcinoma patients. The detection of *Peptostreptococcus* sp. and *Bacteroides* sp. was performed by PCR and Sanger sequencing technique. Phylogenetic analysis of positive samples was carried out to identify the species of detected bacteria. The percent values for *Peptostreptococcus* positive samples were 0, 5.9, 18.2 and 13.2 in each of normal, colitis, precancerous polyps and CRC, respectively. While the per cent values for *Bacteroides* positive samples were 16, 29.4, 36.8 and 23.7 in each of normal, colitis, precancerous polyps, and CRC, respectively. Phylogenetic analysis showed that different species of *Bacteroides* were found in the samples, while only one strain, *Peptostreptococcus anaerobium* NCTCT 11460, was identified in all positive samples. The present study provides new insights into the association of *Peptostreptococcus* sp. and *Bacteroides* sp. with ulcerative colitis, precancerous polyps and colorectal carcinoma in the Iraqi population. Further studies are needed to elucidate the exact role of these bacteria in the pathogenesis of these diseases and to develop novel therapeutic strategies.

Key words: *Peptostreptococcus, Bacteroides*, ulcerative colitis, precancerous polyps, colorectal carcinoma, phylogenetic analysis

INTRODUCTION

Ulcerative colitis (UC) and colorectal carcinoma (CRC) are chronic inflammatory diseases affecting the colon and rectum with a high incidence rate worldwide (Nguyen *et al.*, 2020). Precancerous polyps are abnormal growths in the colon that can develop into cancer if left untreated. The pathogenesis of UC, precancerous polyps and CRC involves complex interactions between genetic and environmental factors, including the microbiota (Dejea *et al.*, 2014; Park *et al.*, 2018; Drewes *et al.*, 2022;). Recent studies have reported alterations in the gut microbiota composition and diversity in patients with UC and CRC, compared to healthy individuals (Gagnière *et al.*, 2016; Wong and Yu, 2019). Several bacterial species have been associated with UC and CRC, including *Peptostreptococcus* sp. and *Bacteroides* sp. (Russo *et al.*, 2018; Cheng *et al.*, 2020; Xu *et al.*, 2020). *Peptostreptococcus* sp. and *Bacteroides* sp. are anaerobic Gram-negative bacteria that are commonly found in the gut microbiota (Hsu *et al.*, 2019). *Peptostreptococcus* sp. is a commensal bacterium that plays a role in the maintenance of gut homeostasis, while *Bacteroides* sp. is involved in the degradation of complex polysaccharides and the production of short-chain fatty acids (SCFAs; Carretta *et al.*, 2021; Zhao *et al.*, 2021). However, under certain conditions, such as dysbiosis, these bacteria can become pathogenic and

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contribute to the development of inflammatory and carcinogenic processes (Genua *et al.*, 2021).

In the Iraqi population, limited information is available regarding the prevalence and phylogenetic analysis of *Peptostreptococcus* sp. and *Bacteroides* sp. among UC, precancerous polyps and CRC patients. Therefore, the present study aimed at investigating the prevalence and phylogenetic analysis of these bacteria among these patient populations using polymerase chain reaction (PCR) and Sanger sequencing techniques.

MATERIALS AND METHODS

The study protocol was approved by the ethical committee of Erbil of Health and Medical Technical College at Erbil Polytechnic University, Erbil, Iraq. All the participants provided written informed consent before inclusion in the study. A total of 100 patients underwent colonoscopy and surgical operation diagnosed with normal colon (25 samples), ulcerative colitis (18 samples), precancerous polyps (19 samples) and colorectal carcinoma (CRC; 38 samples) were recruited from OGD department from three hospitals inside Erbil city from June 2021 until March 2022. All samples were collected from each participant and transferred into sterile tubes containing phospho-buffer saline, PBS. All samples were immediately transported to the laboratory and stored at -80°C until further analysis.

Total genomic DNA was extracted from the fecal samples using the Favor Prep™ Tissue Genomic DNA Extraction Mini Kit (Favorgen Biotech crop, Taiwan), following the manufacturer's instructions. Briefly, the tissue samples were cut up to 25 mg and ground into a micro centrifuge tube. The samples were processed according to the manufacturer's protocol. Each of provided buffers, proteinase K and absolute ethanol, were added to the samples before applying the spin column. The samples underwent centrifugation and ran through column filters using a specific eluting buffer. DNA concentration and purity were measured using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

The bacterial 16S rRNA genes were amplified using specific primers (Table 1). PCR amplification was performed in a 25 µl reaction volume containing (insert reaction components). The PCR amplifications for *Peptostreptococcus* were performed as follows: initial denaturation at 95°C for 5 min, followed by 45 cycles of denaturation at 95°C for 10 s, annealing at 52-56°C for 10 s and extension at 72°C for 10 s. However, the PCR conditions for *Bacetroides* 16S rRNA gene were 95°C for 10 min, followed by 40 cycles of 95°C for 20 s and 60°C for 1 min. The PCR products were visualized on a 2% agarose gel electrophoresis. The positive PCR products were sent to the Molecular Genetics Laboratory in Zheen International Hospital, Erbil, for 16S rRNA sequencing using the same primer. The obtained data were compared to the reference 16S rRNA sequences at the NCBI database via applying nucleotide BLAST. Phylogenetic analysis was performed based on the 16S rRNA nucleotide sequences using Molecular Evolutionary Genetic Analysis (MEGA11) software. The sequence alignment and Phylogenetic analysis were conducted based on the Tamura-Nei model and the bootstrap values were calculated from 1000 replicates.

RESULTS AND DISCUSSION

A total of 100 biopsy samples were collected from 25 healthy controls, 18 UC patients, 19 PP patients and 38 CRC patients. The prevalence and phylogenetic analysis of *Peptostreptococcus* sp. and *Bacteroides* sp. were evaluated in all samples using PCR and Sanger sequencing techniques.

The percentage of *Peptostreptococcus* positive samples was found to be 10.5, 30.6, 42.9 and 53.3 in the healthy control, UC, PP and CRC groups, respectively (Fig. 1). On the other hand, the percentage of *Bacteroides* positive samples was found to be 64.5, 83.3, 94.6 and 90.0 in the healthy control, UC, PP and CRC groups, respectively (Fig. 2).

Molecular detection of *Peptostreptococcus* sp.

Table 1. Primers used for amplification of specific genes in both *Peptostreptococcus* and *Bacetroides*

Primer name	Forward sequence	Reverse sequence	Band size
16S rRNA (*Bacteroides*)	TGGACTGCAACTGACACTGA	GCCGCTTACTGTATATCGCA	(115 bp)
Peptostreptococcus	CTG GTG GATAGGAGGCAAAG	CCA CAA TATTGG CAT TTG GA	(162 bp)

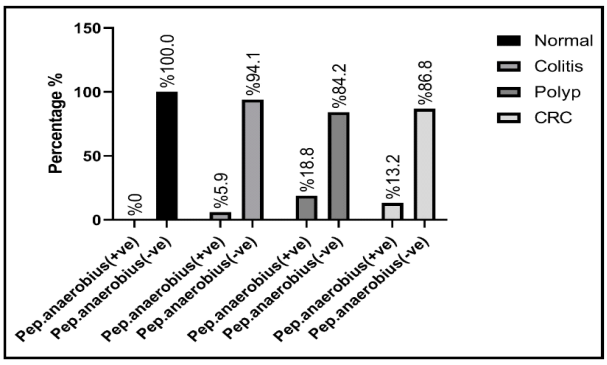

Fig. 1. Per cent values for *Peptostreptococcus* positive samples in each of normal, colitis, precancerous polyps and CRC.

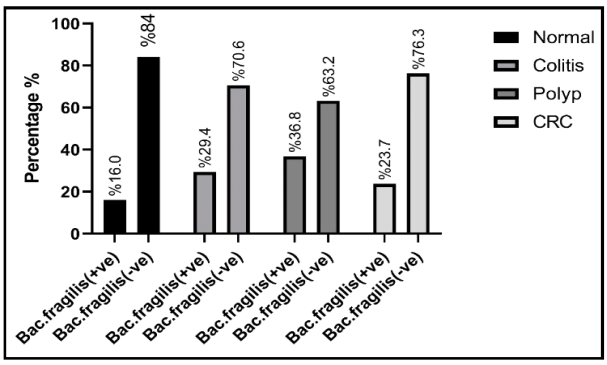

Fig. 2. Per cent values for *Bacteroides* positive samples in each of normal, colitis, precancerous polyps and CRC.

and *Bacteroides* sp. was confirmed by gel electrophoresis of PCR products, which revealed positive bands at the expected sizes (Fig. 3). Sanger sequencing of the PCR products was performed, and the resulting sequences were analyzed using the NCBI nucleotide blast database. The results confirmed the presence of *Peptostreptococcus* sp. and *Bacteroides* sp. in the positive samples (Fig. 4).

Peptostreptococcus sp. and *Bacteroides* sp. are anaerobic bacteria that are commonly found in the human gut microbiota. *Peptostreptococcus* sp. has been reported to have both beneficial and pathogenic effects on the host, depending on the species and context. *Bacteroides* sp. has been implicated in the pathogenesis of UC, PP and CRC through its ability to produce short-chain fatty acids (SCFAs) and lipopolysaccharides (LPS) that can modulate the host immune response and promote inflammation (Ramadass and Catz, 2016). These findings are consistent with previous studies that reported an increase in the abundance of *Peptostreptococcus* sp. and *Bacteroides* sp. in the fecal microbiota of UC, PP and CRC patients (Ananthakrishnan, 2015a, b; Rojas-Tapias *et al.*, 2017).

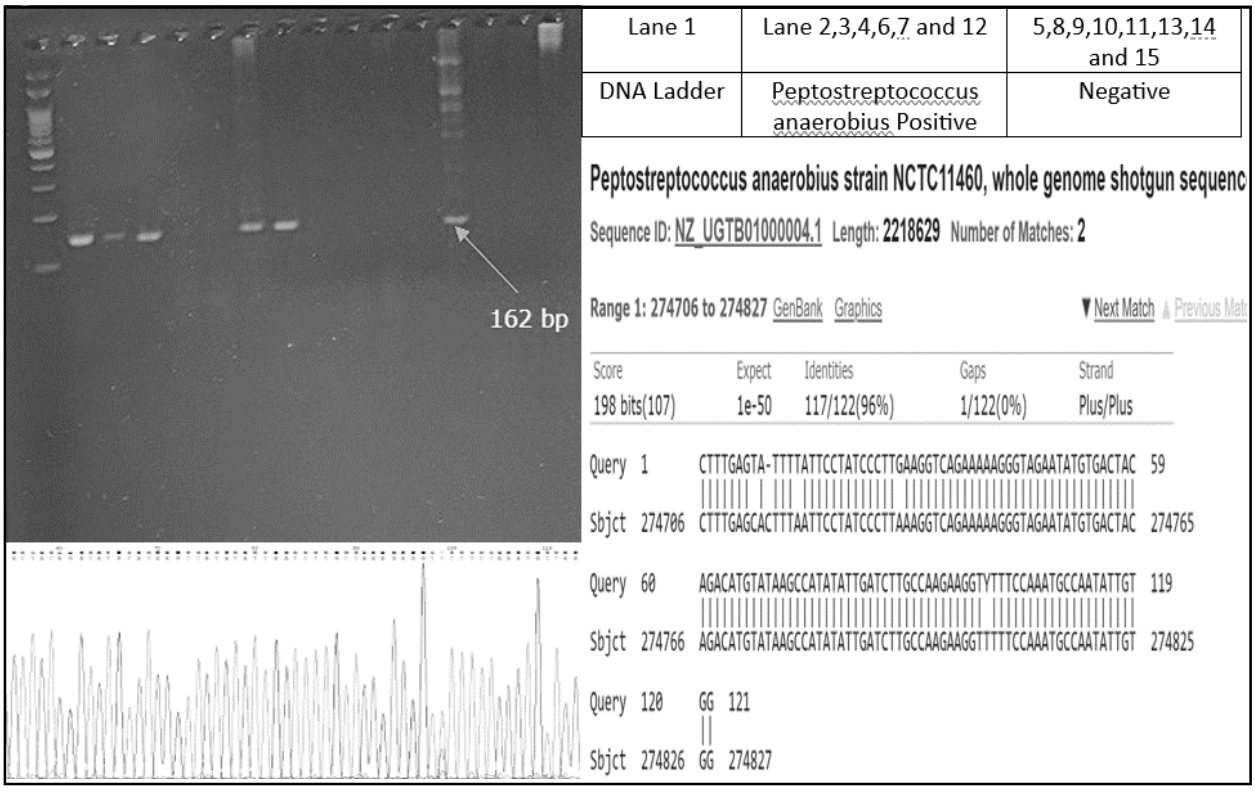

Fig. 3. Gel electrophoresis and NCBI nucleotide blast sequencing results for *Peptostreptococcus* PCR products.

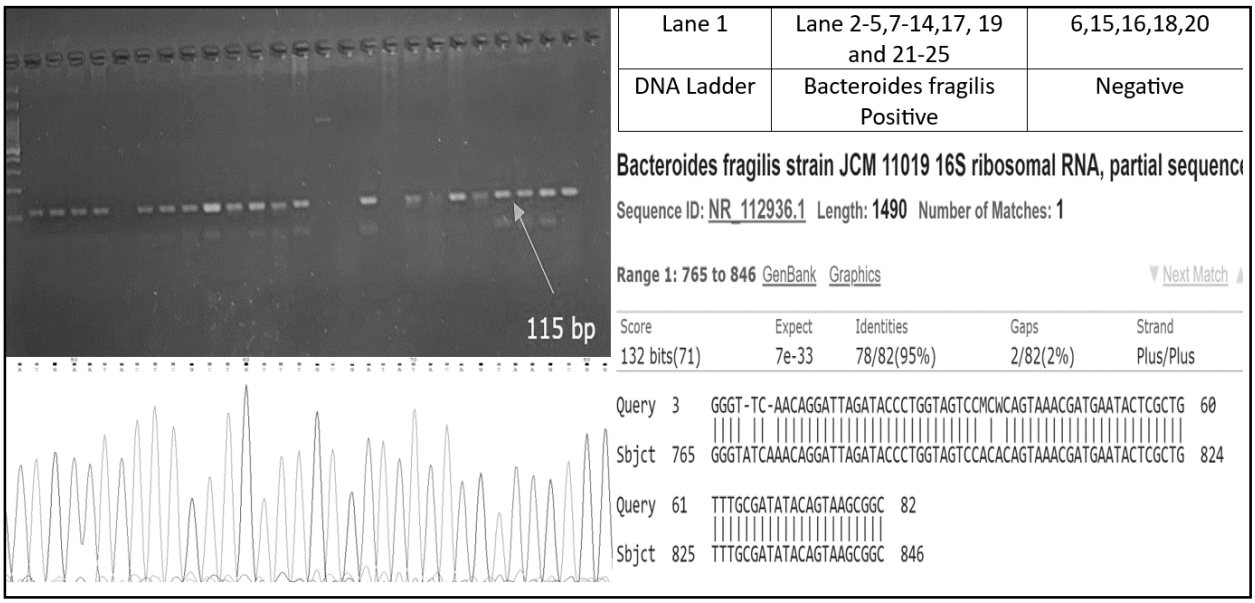

Fig. 4. Gel electrophoresis and NCBI nucleotide blast sequencing results for *Bacteroides* PCR products.

Phylogenetic analysis of *Peptostreptococcus* sp. and *Bacteroides* sp. was performed using the maximum likelihood method based on the 16S rRNA gene sequences. The analysis revealed that all *Peptostreptococcus* positive samples contained *Peptostreptococcus anaerobium* strains (Fig. 5), while the *Bacteroides* positive samples contained different species (Fig. 6).

The phylogenetic analysis of *Peptostreptococcus* sp. and *Bacteroides* sp. revealed the presence of different species in the positive samples. All *Peptostreptococcus* positive samples contained *Peptostreptococcus anaerobius* strains, which have been reported to be involved in the development of periodontal disease and septicemia (Cohen *et al.*, 2022). The *Bacteroides* positive samples contained different species, including *B. vulgatus*, *B. fragilis* and *B. uniformis*, which have been previously implicated in the pathogenesis of UC, PP and CRC (Dejea *et al.*, 2014; Mukhopadhya *et al.*, 2015; Chu *et al.*, 2016).

The results of this study suggest that *Peptostreptococcus* sp. and *Bacteroides* sp. may play a role in the pathogenesis of UC, PP and CRC in the Iraqi population. Further studies are needed to investigate the mechanisms by which these bacteria contribute to the development and progression of these diseases. Moreover, future studies should focus on the functional characterization of the microbial communities associated with UC, PP and CRC to identify potential therapeutic targets for the prevention and treatment of these diseases. In

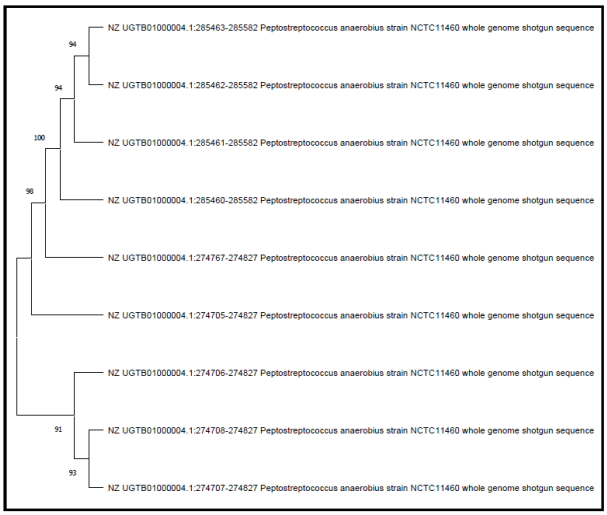

Fig. 5. The phylogenetic tree of *Peptostreptococcus anaerobium* based on 16S rRNA gene sequencing.

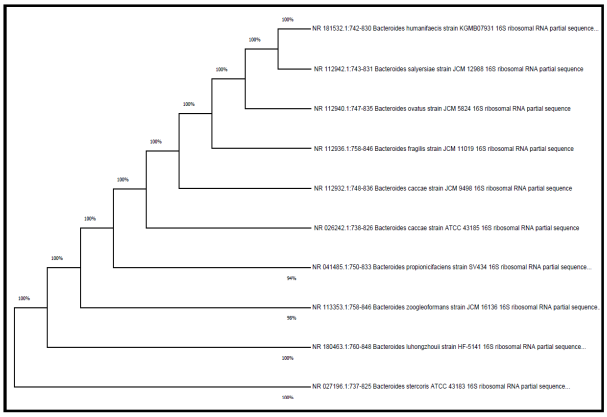

Fig. 6. Phylogenetic tree of *Bacteroides anaerobium* based on 16S rRNA gene sequencing.

conclusion, this study provides evidence of a higher prevalence of *Peptostreptococcus* sp. and *Bacteroides* sp. in UC, PP and CRC patients compared to healthy controls in the Iraqi population. The findings of this study have important implications for the diagnosis and management of these diseases.

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